



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) **EP 1 325 943 A2**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
09.07.2003 Bulletin 2003/28

(51) Int Cl.7: **C09B 61/00, A23L 1/30,**
A23L 1/275

(21) Application number: **03000137.4**

(22) Date of filing: **03.01.2003**

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IT LI LU MC NL PT SE SI SK TR
Designated Extension States:
AL LT LV MK RO

(30) Priority: **08.01.2002 JP 2002001824**

(71) Applicant: **Riken Vitamin Co., Ltd.**
Chiyoda-ku, Tokyo 101-8370 (JP)

(72) Inventors:
• **Sadano, Shin,**
Riken Vitamin Co. Ltd. Kyoto Factory
Kameoka-shi, Kyoto 621-0007 (JP)

• **Harada, Koichi,**
Riken Vitamin Co. Ltd. Kyoto Fact.
Kameoka-shi, Kyoto 621-0007 (JP)
• **Sonoda Tadamichi,**
Riken Vitamin Co. Ltd. Kyoto Fy
Kameoka-shi, Kyoto 621-0007 (JP)

(74) Representative: **Best, Michael, Dr. et al**
Lederer & Keller
Patentanwälte
Prinzregentenstrasse 16
80538 München (DE)

(54) **A method for the production of a lutein-fatty acid ester concentrate**

(57) The present invention relates to a method for the production of a lutein-fatty acid ester concentrate of high purity by dissolving marigold oleoresin in a ketone solvent such as acetone, cooling the solution, and removing therefrom a ketone solvent-insoluble impurity to uniform the quality of oleoresin, dissolving the acetone-soluble portion obtained by removing a ketone solvent from the ketone solvent solution in butanol, cooling the

solution, eliminating a butanol-soluble impurity, and washing the resulting butanol-insoluble with ethanol. Prior to eliminating the butanol-soluble impurity, optionally at least one solvent selected from water, methanol and ethanol can be added to the cooled butanol solution.

EP 1 325 943 A2

Description

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

[0001] The present invention relates to a method for the production of a lutein-fatty acid ester concentrate, more particularly a method of obtaining a lutein-fatty acid ester concentrate of high purity by purifying marigold oleoresin.

2. DESCRIPTION OF THE PRIOR ART

[0002] Lutein-fatty acid esters are a yellow-orange pigment contained in fruits such as oranges, peaches, papayas, prunes and mangos. Lutein-fatty acid esters are also present in many flowers and vegetables, particularly in petals of marigold flowers remarkably. Dried and ground marigold flowers have been used as animal feeds for a long time, and oleoresin which is obtained by extraction of marigold flowers with a solvent has also been widely used as food color in recent years in addition to the above-mentioned application.

[0003] The recent scientific researches have reported that lutein is associated with risk reduction for age-related macular degeneration (AMD) caused by oxidative damage to macular area of retina (Journal of the American Medical Association, 272, 1413-1420, 1994; Investigative Ophthalmology & Visual Science, 38, 1802-1811, 1997, etc.), and that lutein is effective for prevention of arteriosclerosis, prevention of cataract or suppression of carcinogenesis and etc. (British Medical Journal, 305, 335-339, 1992; The American Journal of Clinical Nutrition, 70, 517-524, 1992; American Journal of Epidemiology, 149, 801-809, 1999, etc). As such the use of lutein as a health food and a dietary supplement is also expected.

[0004] Marigold oleoresin is obtained when dried and ground marigold flowers are extracted with a hydrocarbon solvent such as hexane and petroleum ether or with a chlorinated hydrocarbon solvent such as dichloromethane, and the solvent is removed from the extract. But the content of lutein-fatty acid ester as an ester contained in oleoresins produced by the largest producers of marigold extracts is low, usually 14 to 20 % (cf. U.S. Patent No. 6,191,293), and is about 30 to 35 % even in a superior grade type which is partly sold for food.

[0005] Thus, there has been a demand for a lutein-fatty acid ester concentrate of high purity in order to obtain more vivid color tones when it is used as a food color and in order to improve the functional efficiency when it is used as a dietary supplement.

[0006] As to a method of obtaining a lutein-fatty acid ester concentrate of high purity, "Purification of Lutein-Fatty Acid Esters From Plant Materials" (U.S. Patent No. 4,048,203) and "Trans-xanthophyll Ester Concentrates of Enhanced Purity and Methods of Making Same" (U.S. Patent No. 6,191,293) have been disclosed.

[0007] The former describes a method which comprises precipitating lutein-fatty acid esters from oleoresin which is dissolved in hot alkanol containing not more than four carbon atoms by cooling the solution, removing alkanol therefrom by filtration and drying the lutein-fatty acid ester under vacuum. However, the purity of the lutein-fatty acid ester concentrate obtained by this method is 51%, which cannot be considered a high purity.

[0008] The latter describes a method of obtaining a purified trans-xanthophyll ester by contacting coronals (or flowers) of marigold flowers with a hydrocarbon solvent, removing therefrom plant residue after sufficient extraction, evaporating the extract to obtain oleoresin, admixing the resulting oleoresin with an alcohol at ambient temperature and removing the alcohol containing impurity and cis-xanthophyll ester from the mixture.

[0009] By this method, however, the purity of lutein-fatty acid ester in the resulting oleoresin varies widely depending on the quality of the raw material, i.e. coronals (or flowers) of marigold flowers, and therefore, the purity of lutein-fatty acid ester in a concentrate obtained by purifying the said oleoresin also varies widely. For this reason, control of the raw material is necessary to obtain a lutein-fatty acid ester concentrate of high purity by this method, and as such it is difficult to stably obtain a lutein-fatty acid ester concentrate of high purity when most of the commercially available oleoresins are used as a starting material.

SUMMARY OF THE INVENTION

[0010] An object of the present invention is to provide a production method by which a lutein-fatty acid ester concentrate of high purity can be stably obtained even when oleoresin containing lutein-fatty acid esters of low purity is used as a starting material, without being affected by variation of the quality, particularly the content of lutein-fatty acid esters, of marigold oleoresin.

[0011] The present inventors have found that, as a result of diligent studies to solve the above-mentioned problems, a lutein-fatty acid ester concentrate of high purity is stably obtained by dissolving oleoresins in a ketone solvent such as acetone, cooling the solution, and removing therefrom a ketone solvent-insoluble impurity such as phospholipid to

uniform the quality of oleoresins. Furthermore, preferably, the ketone solvent-soluble portion obtained by removing a ketone solvent from the ketone solvent solution is dissolved in butanol, the solution is cooled, and a butanol-soluble impurity is eliminated. Furthermore, preferably, the resulting butanol-insoluble is washed with ethanol to eliminate an ethanol-soluble impurity. Prior to eliminating the butanol-soluble impurity, optionally at least one solvent selected from water, methanol and ethanol can be added to the cooled butanol solution. A process in which a butanol-insoluble which is isolated from a butanol solution containing lutein-fatty acid ester obtained by processing marigold oleoresin is washed with ethanol also yields a lutein-fatty acid ester concentrate of high purity. The present inventors have accomplished the present invention after further studies based on the finding.

[0012] Thus, the present invention is directed to the following (1) to (13).

(1) A method for the production of a lutein-fatty acid ester concentrate of high purity, which comprises a step (a) of dissolving marigold oleoresin in a ketone solvent, preferably acetone, cooling the solution and removing therefrom a ketone solvent-insoluble impurity.

(2) Said method (1) which further comprises a step (b) of dissolving a ketone solvent-soluble portion obtained by removing a ketone solvent from the ketone solvent solution, in butanol, preferably n-butanol, cooling the solution and eliminating a butanol-soluble impurity.

(3) Said method (2) which further comprises the addition of at least one solvent selected from water, methanol, ethanol and a mixture thereof, to the cooled butanol solution, prior to eliminating the butanol-soluble impurity.

(4) Said method (2) or (3) which further comprises a step (c) of washing the butanol-insoluble of step (b) with ethanol to eliminate an ethanol-soluble impurity.

(5) Said method (4) which further comprises removing the residual solvent after step (c).

(6) A method for the production of a lutein-fatty acid ester concentrate of high purity, which comprises a step (c) of washing a butanol-insoluble which is isolated from a butanol solution containing lutein-fatty acid ester obtained by processing marigold oleoresin with ethanol.

(7) Said method (6), wherein the solvent of the butanol solution comprises butanol, preferably n-butanol, and optionally one or more solvents selected from water, methanol, ethanol and a mixture thereof.

(8) Said method (6) or (7), which further comprises removing a residual solvent after step (c).

(9) A composition comprising not less than 70 wt.% of lutein-fatty acid esters.

(10) Said composition (8), obtainable by the aforesaid methods (1) to (8).

(11) A health food or a dietary supplement comprising said composition (9) or (10).

(12) Use of said composition (9) or (10) for the production of health food or a dietary supplement.

(13) A method of making a health food or a dietary supplement which comprises incorporating said composition (9) or (10) into a health food or dietary supplement.

DETAILED DESCRIPTION OF THE INVENTION

[0013] Marigold oleoresin used in the present invention is a solid or a viscous liquid having yellow-orange/yellowish-brown color, which is obtained by drying flowers of marigold which is a member of the Compositae family (*Tagetes recta* WILLD.), grinding the dried product, optionally converting into pellet, extracting with an organic solvent, usually hexane, and removing the solvent from the extract. Its main component is lutein (one of xanthophylls) dipalmitate.

[0014] According to the method of the present invention, marigold oleoresin (also called oleoresin for short) is dissolved in a ketone solvent. A ketone solvent used here includes a ketone solvent containing 3 to 9 carbon atoms such as acetone, methylethylketone and diethylketone, among which acetone is preferable. The amount of acetone is preferably about 0.5 to 10 parts by weight, more preferably about 2 to 3 parts by weight, relative to 1 part by weight of marigold oleoresin which is the raw material.

[0015] A mixture of oleoresin and acetone is stirred for about 0.5 to 1 hour at about 40 to 55°C, preferably at about

45 to 50°C while keeping moderate refluxing. Next the mixture is slowly cooled to about 10 to 30°C, preferably about 15 to 25°C over a period of usually about 2 to 4 hours. The cooled mixture is filtered through filter paper or filter fabric with filter aid, if necessary, such as diatomite. The filtrate is evaporated under reduced pressure and acetone is removed therefrom to obtain a solid or a viscous liquid consisting of acetone-soluble portion in oleoresin.

[0016] According to the method of the present invention, acetone-soluble portion in oleoresin is dissolved in butanol. Examples of butanol used here are n-butanol, i-butanol, sec-butanol and tert-butanol, among which n-butanol is preferable. The amount of butanol is preferably about 1 to 5 parts by weight, more preferably about 2 to 3 parts by weight relative to 1 part by weight of the said soluble portion.

[0017] The mixed solution of the said soluble portion and butanol is stirred for about 0.5 to 1 hour at about 40 to 80°C, preferably at about 45 to 55°C. Next the mixture is slowly cooled to about 0 to 20°C, preferably about 3 to 10°C over a period of usually about 2 to 5 hours. The cooled mixture is filtered through filter paper or filter fabric to recover a butanol-insoluble solid, and the filtrate containing a butanol-soluble impurity is discarded.

[0018] According to the method of the present invention, one or more solvents selected from the group consisting of water, methanol, ethanol and a mixture thereof can be added, preferably while stirring, to the above cooled mixture. By adding these solvent(s), an insoluble solid is precipitated rapidly, by which operating efficiency can be improved. The amount of water to be added here is preferably about 0.01 to 1 part by weight, more preferably about 0.05 to 0.5 part by weight relative to 1 part by weight of the butanol used; the amount of methanol is preferably about 0.01 to 5 parts by weight, more preferably about 0.1 to 1 part by weight relative to 1 part by weight of the butanol used; and the amount of ethanol is preferably about 0.01 to 10 parts by weight, more preferably about 0.5 to 5 parts by weight relative to 1 part by weight of the butanol used. Although the temperature of solvent(s) to be added here may be ambient temperature, the same temperature as the cooled mixture, if possible, is preferable. The mixture consisting of mixed solvents is filtered through filter paper or filter fabric to recover a solid which is insoluble in the mixed solvent, and the filtrate containing an impurity which is soluble in the mixed solvent is discarded.

[0019] According to the method of the present invention, the butanol-insoluble or the mixed solvent-insoluble wherein butanol is a (preferably main) component is washed with ethanol. The amount of ethanol is preferably about 5 to 80 parts by weight, more preferably about 10 to 40 parts by weight relative to 1 part by weight of the said insoluble. By washing with ethanol, butanol or the mixed solvent consisting mainly of butanol which is adhered to the surface of the said insoluble is eliminated, and consequently the removal of solvent in finishing process becomes easier.

[0020] A mixture of the said insoluble and ethanol is stirred for about 0.5 to 2 hour(s), preferably about 1 to 1.5 hour at ambient temperature. Next the mixture is filtered through filter paper or filter fabric to recover a lutein-fatty acid ester concentrate, and the filtrate containing an ethanol-soluble impurity is discarded.

[0021] Finally, the residual solvent is removed from the purified lutein-fatty acid ester concentrate by evaporation using, for example, a vacuum shelf dryer at a temperature of, for instance, lower than 50°C preferably in the atmosphere of nitrogen gas.

[0022] The purity of lutein-fatty acid ester contained in the lutein-fatty acid ester concentrate which is obtained by the method according to the present invention is about not less than 70%, and that with about more than 80% purity is frequently obtained. As a result of this, the utilization range as a food color is broadened due to the vivid color tone, and it becomes possible to blend a lutein fatty-acid ester concentrate having high concentration into a dietary supplement by micro-encapsulation.

WORKING EXAMPLE

[0023] The following examples specifically illustrate the present invention.

A METHOD OF MEASURING THE CONTENT OF LUTEIN-FATTY ACID ESTER

[0024] About 0.1g of test sample was accurately measured and dissolved in hexane to make a 100ml solution. It was diluted with hexane so that the resultant solution had absorbance ranging from 0.3 to 0.7, and then the maximum absorbance of the diluted solution around 445nm was measured by a spectrophotometer. The content of lutein-fatty acid ester was calculated by the following formula.

$$\text{Content(\%)} = ((\text{Absorbance} \div \text{Weight of Sample}) \times \text{Dilution Ratio}) \div 1,394 \times 100$$

Note: 1,394 is absorbance coefficient at 445nm

EXAMPLE 1

[0025] 107g of marigold oleoresin containing 26wt% of lutein-fatty acid ester was mixed with 214ml of acetone and dissolved at 50°C under heating. Next the solution was slowly cooled to 20°C over a period of about 3 hours and the resulting insoluble was eliminated by filtration, and then the filtrate was evaporated under reduced pressure to obtain a concentrate of the acetone-soluble portion. 300g of n-butanol was added to this concentrate (100g), and then the solution was dissolved at 45°C under heating and slowly cooled to 4°C over a period of about 4.5 hours.

[0026] After further keeping it at 4°C for 16 hours, the resulting insoluble was filtered through filter paper. The solid which remained was mixed with 800ml of ethanol, and the mixture was stirred, dispersed, washed at ambient temperature, and then filtered again through filter paper. The resulting solid was dried under vacuum to remove residual solvent, whereby 8.3g of a concentrate containing 89wt% of lutein-fatty acid ester was obtained.

EXAMPLE 1a

[0027] 107g of marigold oleoresin as used in Example 1 was mixed with 214ml of acetone and dissolved at 50°C under heating. The solution was slowly cooled to 20°C over a period of about 3 hours and the resulting insoluble was eliminated by filtration, and then the filtrate was evaporated under reduced pressure to obtain a concentrate of acetone-soluble portion.

[0028] Next, according to the method mentioned in Example 1 of USP 6,191,298, this concentrate (100g) was stirred for 3 hours with 200g of isopropanol at ambient temperature to obtain a suspension. The suspension was filtered through filter paper and the solid left on the filter paper was dried under vacuum at ambient temperature to eliminate residual solvent, whereby 34.6g of a solid containing 44wt% of lutein-fatty acid ester was obtained.

COMPARATIVE EXAMPLE 1

[0029] According to the method mentioned in Example 1 of USP 6,191,298, 100g of marigold oleoresin as used in Example 1 was stirred for 3 hours with 200g of isopropanol at ambient temperature to obtain a paste-like suspension. The suspension was filtered through filter paper and the solid left on the filter paper was dried under vacuum at ambient temperature to eliminate residual solvent, whereby 35.6g of a concentrate containing 37wt% of lutein-fatty acid ester was obtained.

EXAMPLE 2

[0030] 106g of marigold oleoresin containing 22wt% of lutein-fatty acid ester was mixed with 212ml of acetone and dissolved at 50°C under heating. Next the solution was slowly cooled to 20°C over a period of about 3 hours and the resulting insoluble was eliminated by filtration, and then the filtrate was evaporated under reduced pressure to obtain a concentrate of acetone-soluble portion. 400g of n-butanol was added to this concentrate (100g), dissolved at 45°C under heating, and slowly cooled to 4°C over a period of about 4.5 hours.

[0031] 26.3g of water was added thereto and the resulting insoluble was filtered through filter paper. The solid obtained as above was mixed with 800ml of ethanol for washing by stirring and dispersion at ambient temperature, and then filtered again through filter paper. The resulting solid was dried under vacuum to remove residual solvent, whereby 15.9g of a concentrate containing 82wt% of lutein-fatty acid ester was obtained.

EXAMPLE 2a (No acetone treatment)

[0032] 300g of n-butanol was added to 100g of marigold oleoresin as used in Example 2, dissolved at 45°C under heating, and slowly cooled to 4°C over about 4.5 hours. 32.6g of water was added thereto and the resulting insoluble was filtered through filter paper. The solid obtained as above was mixed with 800ml of ethanol for washing by stirring and dispersion at ambient temperature, and then filtered again through filter paper. The resulting solid was dried under vacuum to remove residual solvent, whereby 36.4g of a concentrate containing 46wt% of lutein-fatty acid ester was obtained.

EXAMPLE 2b (No butanol treatment)

[0033] 106g of marigold oleoresin as used in Example 2 was mixed with 212ml of acetone and dissolved at 50°C under heating. Next the solution was slowly cooled to 20°C over about 3 hours and the resulting insoluble was eliminated by filtration, and then the filtrate was evaporated under reduced pressure to obtain a concentrate of acetone-soluble portion. This concentrate (100g) was mixed with 2,010ml of ethanol for washing by stirring and dispersion at ambient

temperature, and filtered again through filter paper. The resulting solid was dried under vacuum to remove the residual solvent, whereby 35.2g of a concentrate containing 52wt% of lutein-fatty acid ester was obtained.

EXAMPLE 3

[0034] 214g of methanol was used in place of 26.3g of water in Example 2, and 18.7g of a concentrate containing 73wt% of lutein-fatty acid ester was obtained.

EXAMPLE 4

[0035] 1,250g of ethanol was used in place of 26.3g of water in Example 2, and 18.4g of a concentrate containing 77wt% of lutein-fatty acid ester was obtained.

INDUSTRIAL APPLICABILITY

[0036] The method of the present invention provides a lutein-fatty acid ester concentrate of high purity, and as a result of this, the utilization range as a food color is broadened due to the vivid color tone of a lutein-fatty acid ester concentrate, and it becomes possible to blend a lutein-fatty acid ester concentrate having high concentration into a dietary supplement by micro-encapsulation.

Claims

1. A method for the production of a lutein-fatty acid ester concentrate of high purity, which comprises a step (a) of dissolving marigold oleoresin in a ketone solvent, cooling the solution and removing therefrom a ketone solvent-insoluble impurity.
2. The method according to claim 1, wherein said ketone solvent is acetone.
3. The method according to claim 1 or 2, which further comprises a step (b) of dissolving a ketone solvent-soluble portion obtained by removing a ketone solvent from the ketone solvent solution, in butanol, cooling the solution and eliminating a butanol-soluble impurity.
4. The method according to claim 3, which further comprises the addition of at least one solvent selected from water, methanol and ethanol to the cooled butanol solution, prior to eliminating the butanol-soluble impurity.
5. The method according to claim 3 or 4, which further comprises a step (c) of washing the butanol-insoluble of step (b) with ethanol to eliminate an ethanol-soluble impurity.
6. The method according to claim 5, which further comprises removing the residual solvent after step (c).
7. A method for the production of a lutein-fatty acid ester concentrate of high purity, which comprises a step (c) of washing a butanol-insoluble which is isolated from a butanol solution containing lutein-fatty acid ester obtained by processing marigold oleoresin with ethanol.
8. The method according to claim 7, wherein the solvent of the butanol solution comprises butanol and optionally one or more solvents selected from water, methanol and ethanol.
9. The method according to claim 7 or 8, which further comprises removing a residual solvent after step (c).
10. The method according to any of the preceding claims, wherein said butanol is n-butanol.
11. A composition comprising not less than 70 wt.% of lutein-fatty acid esters.
12. A composition according to claim 11, obtainable by the method according to any of claims 1 to 10.
13. A health food or a dietary supplement comprising a composition of claim 11 or 12.

EP 1 325 943 A2

14. Use of the composition of claim 11 or 12 for the production of health food or a dietary supplement.

15. A method of making a health food or a dietary supplement which comprises incorporating a composition of claim 11 or 12 into a health food or dietary supplement.

5

10

15

20

25

30

35

40

45

50

55

